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The Agricultural Chemical Society of Japan.

President : Umetarō Suzuki.

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2. No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japaneses texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Umetarō Suzuki.

Associate Editors : Kakuji Gotō and Yoshihiko Matsuyama.

ON THE ORGANIC BASES OF THE PUPA OF TUSSAH SILK WORM (ANTHERAEA PERYRI).

By Jirō Katō.

From the Central Laboratory, South Manchuria Railway Co., Dairen, South Manchuria.

(Received March. 8th., 1926)

4 kgs. of powdered pupa of tussah silk worm were extracted with hot water. About 40 litres of the aqueous extract thus obtained were evaporated to about one-tenth of its volume. The precipitate formed on this process consisted mainly of the urate, as was reported previously (The report of the coutral laboratory of S. M. R. Co., No. X.) It was filtered and washed with water. The filtrates and the washings were combined and treated by lead acetate to get rid of impurities. From the filtrate, the lead was removed by passing sulfuretted hydrogen. When the filtrate was neutralized and evaporated down to about 2 litres, about 2 grs. of tyrosine crystallised out. The filtrate from tyrosine was treated with phosphotungstic acid and baryta in the ordinary way.

The free base solution thus obtained was separated into the following fractions :- purine fraction, histidine fraction, arginine fraction and lysine fraction. From these fractions the following bases were isolated :-

Adenine as picrate.	2.8 g.
Hypoxanthine.	0.27 g.
Histidine as dipicrolonate.	0.30 g.
Arginine as picrate.	0.40 g.
Choline as gold salt.	8.50 g.
Betaine as picrate.	present.
Lysine as picrate.	9.51 g.

The content of the bases of this pupa is smaller than that of the silk worm pupa. Any special base, which has not been isolated from the silk worm pupa, was not detected. The cadaverine and putrescine, which K. Katayama isolated from the silk worm pupa, were not met with here. The absence of putrescine and cadaverine, the greater yield of lysine, and the smaller total content of organic bases are the chief difference of this pupa from the silk worm pupa, in regard to organic bases.

THE CHEMICAL PROPERTIES, AND THE NUTRITIVE VALUE OF THE PROTEIN OF ITALIAN MILLET (SETARIA ITALICA, KTH).

By Mitsuyuki KONDO.

From the Imperial Government Institute of Nutrition, Tokyo, Dr. Tadasu Saiki, director.

(Received Feb. 13 th., 1926)

In our country, Italian millet is an important cereal, and ranks next to rice and wheat. In spite of this fact there seems to be lacking a detailed investigation of its protein and of its nutritive value.

The Italian millet seed used in these experiments was polished by the polishing machine of this institute and then powdered.

The analysis gave the following constitution :–

	Air dry matter %	Dry matter %
Moisture	13.57	—
Crude protein	11.12	12.27
Protein	10.36	11.98
Crude fat	5.32	6.16
N-free-extract	63.78	73.79
Crude fibre	1.71	2.01
Crude ash	0.87	1.01

1. Solubility of proteins.

Separation of proteins :– before separating the proteins into fractions, their solubility in different solvents was examined. As solvents, distilled water, 10 % saline solution, 0.2 % alkaline solution, and 10 % alcohol were used. The procedure was as follows :–

15 gr. of the sample were agitated with nearly 800 c.c. of distilled water for 24 hours at room temperature. After filtering and washing, the filtrates and the washings were combined and made up to 1 liter. Then the residue was treated with 10 % saline solution, 0.2 % alkaline solution and 70 % alcohol successively in the same way as stated above. Of these four filtrates, the albuminous nitrogen was determined.

	Dry matter %	Total N %
Total N of this sample	1.98	100.00

Water soluble N	0.37	21.85
10% saline solution sol. N	0.43	25.48
0.2% alkaline " " "	0.52	30.15
70% alcohol " " "	0.96	56.21

Subsequently, the samples were extracted by these solvents separately and the following results were obtained :–

Sample gr.	Total N gr	Solvent (200c.c.)	Temp.	Time hrs.	N-extracted gr.	%
3.06	0.0544	Water	40°C	5	0.0228	41.91
2.64	0.0469	10 % saline solution	40°	5	0.0114	24.48
3.81	0.0678	0.2 % alkaline "	40°	5	0.0275	40.62
2.32	0.0413	70 % alcohol "	60°	5	0.0209	50.60

Note : These extractions were made under an elevated temperature.

From these results, it is evident that Italian millet contains mainly the 70 % alcohol soluble protein and 0.2 % alkaline soluble protein, so the author then separated these two proteins.

2. The separation of the alcohol soluble protein.

300 gr. of the sample were heated with 5 times its weight of 70 % alcohol in a triple necked flask and agitated for 6 hours at 60°C and immediately filtered. The yellow filtrate was evaporated in a vacuum to get rid of the greater part of the alcohol. The residue, on pouring into cold water gave the precipitation of the protein as a yellow viscous mass. Then after filtering and washing with water, it was dried, powdered and washed with ether. 18 gr. of nearly pure protein were obtained.

3. The separation of alkaline soluble protein.

350 gr. of the sample were mixed with 4 liters of 0.2 % NaOH solution and agitated for 5 hours at room temperature, and allowed to stand for a few hours. In order to effect the filtration as quickly as possible, it was separated by a centrifugal machine (Sharples), and the filtrate was neutralized with acetic acid. The precipitate thus formed was collected, again dissolved in an alkaline solution and precipitated by acetic acid. After washing with water, alcohol, and ether, the dried protein thus obtained weighed 20 gr.

The Nitrogen Distribution in Italian Millet, in its Alkaline Soluble Protein, and Alcohol Soluble Protein.

1. The nitrogen distribution in Italian millet.

The nitrogen distribution of the seven groups of amino acids in this sample was determined directly by the Van Slyke method as follows :

	Dry matter %	Total N %
Total N	2.033	100.01
Hot 20 % HCl soluble N	1.958	96.29
" " " insoluble N	0.075	3.71
Humin N	0.052	2.54
Amide N	0.244	12.03
Phosphotungstates N	0.605	29.78
Cystine N	0.045	2.21
Arginine N	0.197	9.71
Histidine N	0.240	11.81
Lysine N	0.123	6.05
Amino N	0 508	25.02
Non-amino N	0.097	4.76
Mono amino N	1.003	49.43

2. The nitrogen distribution in the alkaline soluble protein.

The method used was the same as in the previous analysis. The nitrogen distribution in this protein was as follows :–

	Dry matter %	Total N %
Totel N	10.020	100.00
Hot 20 % HCl soluble N	5.681	59.69
" " " insoluble N	4.039	40.31
Humin N	0.555	5.54
Amide N	1.162	11.60
Phosphotungstates N	2.373	23.68
Cystine N	0.188	1.88
Arginine N	0.703	7.02
Histidine N	0.944	9.42
Lysine N	0.537	5.36
Amino N	1.794	17.90
Non-amino N	0.579	5.78
Mono-amino	5.316	53.05

3. The nitrogen distribution in the alcohol soluble protein.

	Dry matter %	Total N %
Total N	13.120	100.00
Hot 20 % HCl soluble N	12.372	94.30
" " " insoluble N	0.748	5.70
Humin N	1.452	11.07
Amide N	2.354	17.94
Phosphotungstates N	1.846	17.94
Cystine N	0.218	1.66
Arginine N	0.664	5.06
Histidine N	0.749	5.71
Lysine N	0.615	4.69
Amino N	1.249	9.52

Non-amino N	0.997	7.60
Mono-amino N	6.879	52.43

From these results, the Italian millet protein is rich in hexone base-N, and also contains an adequate quantity of lysine N. It is therefore to be regarded to have a great nutritive value.

Feeding Experiment on the Nutritive Value of this Protein.

1. Preparation of the ration.

In order that the ration may contain only the protein content of Italian millet, the millet flour was used, and the deficient constituents other than protein were added, using the analysis of the whole grain as a standard. The deficiencies were supplemented as follows: carbohydrate with starch, fat with butter, which is simultaneously used as a source of vitamin A, and mineral matters with Nelson's salt-mixture, and as a source of vitamin B, a small quantity of oryzanin powder was added. The ratio of the constituents of this ration is as follows :-

Protein	10
Fat	14
Ash	4
Carbohydrate	72

Taking the moisture into consideration, the ration was mixed as follows :-

Italian millst powder	100 gr.
Butter	12 〃
Nelson's salt-mixture	4 〃
Starch	10 〃

This diet was heated with four times its weight of water on the water bath until dextrinized.

2. The course of the feeding experiment.

5 male and 5 female albino rats were fed on this diet from Dec. 3, 1923 to Aug. 7, 1924.

It is evident that this diet containing 10 % protein is sufficient to maintain the normal growth of the rats, though there were some increases and decreases of body weight during this period. The average body weight of the young male rats was 58 gr., and this gained the body weight of 306 gr.; while the young female rats increased from the average weight of 59 gr. to 253 gr.

Notwithstanding the fact that the protein of Italian millet is vegetable protein, still it proves to be very favorable for growth.

STUDIES ON THE CELLULOSE.

(PUBLICATION ON CELLULOSE NO. I.)

By Arao ITANO.

Division of Chemistry and Microbiology, Ohara Institute for Agricultural Research, Kurashiki, Okayama-Ken, Japan.

(Received April 28th., 1926)

This paper presents a preliminary report on the bio-physico-chemical problems which are in course of investigation on the cellulose.

Cellulose is one of the most important carbohydrates from many standpoints, namely its wide distribution in nature and its usefulness in manufacturing. Especially in agriculture, the important rôle in maintenance of proper soil conditions has been well known. It however has been very little known as to its exact chemical nature on account of its peculiar properties. Through the thermodynamical studies together with the investigations on the process of decomposition, some additional information may be obtained.

Cellulose is decomposed by comparatively few microorganisms in spite of the enormous amounts of cellulose produced and destroyed every year on earth. Among the few organisms isolated, the anaerobic grouph has been investigated[1] to some extent as to their activity. But the others received comparatively little attention in recent years.[2] An aerobic, thermophilic cellulose fermenter has been investigated here in regard to their energetics, intermediate and end-products of the fermentation.

In regard to the studies of energetics on the members of Schizophyta, only few literature is available. Since Rubner[3] investigated the subject, there has been very little work done. Especially on the soil microorganisms, only few investigation are found on record.[4]

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(1) Omelianski, Centralbl. f. Bakt., II, 1902, 193.

(2) C. van Iterson, Centralbl. f. Bakt., II, **23**, 689;
Hutchinson & Clayton, J. Agr. Science, **9**, 143;
McBeth & Scales, U. S. Dep't Agr. B. P. I. Bull., 266;
Viljoin & others, J. Agr. Science, **16**, 1926, 1; and others.

(3) M. Rubner, Archiv. f. Hygine, **48**, 1904, 260. **57**, 1906, 161.
A. Putter, Vergleichende Physiologie, 1911, **37**.

(4) S. Winogradsky, Bot. Zeitung, **45**, 1187, 489;
N. L. Sohngen, Centralbl. f. Bakt., **15**, 1906, 513;
F. H. van Suchtelen, Centralbl. f. Bakt., 2, **58**, 1923.

I. Thermodynamics Involved in the Cellulose Decomposition.

As it is well known, the plants synthesize the cellulose out of carbon dioxide and water, and when the plant residues find their way into the soil, they are decomposed by,

1. the enzyme cytase,
2. the anaerobic microorganisms,
3. the aerobic microorganisms.

Of course in the last two cases, the action may be due to the enzyme secreted by the organism, but it has not so far been demonstrated. Again it is not probable that the cytase survives the temperature of 65°C for so long time as the thermophilic cellulose fermenter do unless some special conditions exist. As the end products of decomposition, the carbon dioxide and water are given off. This transformation involves not only bio-chemical but physical process namely the thermodynamics or energetics.

Then, in the process of formation of cellulose, certain amount of energy absorbed or endothermic reaction takes place, and the exothermic reaction follows in course of decomposition. This transformation of energy taking place in soil is very important from the soil microbiological standpoint. That is while the plants utilize the sun's energy in the process of synthesis, the microorganisms must find the energy supply to elsewhere, and the source became more clearly understood by the study of fermentation by Rubner[3] and few others.

In investigating this phase of problem on the cellulose, it was found very difficult on account of the lack of exact chemical knowledge of cellulose and also the nature of the process involved. For instance, in looking up the literature on the heat of formation and also the heat of combustion, the numerical value given by the different authors is somewhat different. Examining the data collected in the light of energy equation of cellulose and comparing it with that of glucose, one finds that

For

Glucose, $C_6H_{12}O_6 + 12\,O = 6CO_2 + 6H_2O + 677.2$ Kcal[5]

$-x \quad - \quad 0 \quad = -565.8 - 409.8 + 674.$ " [6]

and for,

Cellulose, $C_6H_{10}O_5 + 12\,O = 6CO_2 + 5H_2O + 680.4$ Kcal [5]

$-x \quad - \quad 0 \quad = -565.8 - 341.5 + 680.0$ " [6]

From these equations, the heat of formation[7] is calculated and found

(5) R. Biedermann, Chemiker-Kalender.

(6) W. Nernst, Theoretical Chemistry, 6th edition;

(7) F. H. Getman, Outlines of Theoretical Chemistry, 3rd Edition.

as follows: i. e., calculating for x,

For
Glucose, 301.6 and Cellulose, 227.3 Kcal.

From the heat of formation thus calculated, one may be informed very approximately as to the quantity of energy involved in the synthesis, and also from the heat of combustion, we find the maximum energy could be liberated on its complete oxidation. Further, from the calorific value determined, the constitution of cellulose may possibly be ascertained more accurately and clearly than has been known.[7] This phase of the investigation will be reported in detail in near future.

In the light of the energy equation, the possible process of cellulose decomposition may be noted as follows, assuming that an initial step is hydrolysis:

$$O_6H_{10}O_5 + H_2O = C_6H_{12}O_6 + x \text{ cal.}$$

In the above equation, the value for x cal. would be very small, and the nature of $C_6H_{12}O_6$ resulted differ by different process. It can be any one of the monosaccharide, namely mannose, galactose, glucose etc. So far as the author is aware, the products of aerobic, cellulose decomposition have not been studied to any extent.

Once the cellulose is hydrolysed into monosaccharide, it offers many possibilities, such as well known reaction, intermolecular etc.,

By an intermolecular reaction:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 22 \text{ Kcal.}^{(3)} = 2C_3H_6O_3 + 15 \text{ Kcal.}^{(3)}$$

By further oxidation:

$$C_2H_5OH + 2O = CH_3COOH + H_2O + 115 \text{ Kcal.}$$

Besides these well known, possible reactions, the products produced first may undergo the further decomposition in course of investigation. Consequently the exact processes, and the nature of the products in the cellulose decomposition may never be found out exactly. However an attempt is made here to study the products of aerobic decomposition and thermodynamics involved, and it is hoped to obtain some additional information on the subject, which will be reported in near future.

II. Thermophilic Cellulose Fermenter.

An organism which has received a special attention in the investigation here is an aerobic thermophilic bacteria of which description will appear later.

This organism has been investigated in view of the fact that the aerobic cellulose fermenter has received very little attention in past, although I believe

it plays a very important rôle in the process and subsequently in agriculture. Thermophilic nature of this organism enables it to work at high temperature which is often reached in composting, where the temperature rises up to 75°C often.

III. Practical Application.

In recent years, the study on the rôle of organic matter in soil fertility has become acute.[8] Schreiner[9] stated "It is only by continually supplying organic matter that the soil-forming, soil fertility promoting, dynamic changes can continue to go on unchecked and undiminished, liberating ammonia and other compounds, *supplying energy* for bacterial life and furthering nitrification and nitrogen fixation". Further the same author stated, "If we would understand soil fertility as influenced by *organic manures*, green manures, and good farming methods we must study not so much the organic content, except it be as a key to these *dynamic factors*, but the organic chemical changes themselves which affect soil fertility must be clearly worked out. In this field of research activity much remains to be done".

While the scientific investigations named previously have been in progress, the practical experiments have been carried out during the last one year and half to obtain a desirable organic farm-yard manure, or composting on the basis of scientific information available. A brief abstract of the method will be given below and the detail description will be published later:

1. The materials used;

   Straw, weeds, garbage, street sweeping, plant residue, rice hask, human manure, and any other organic waste materials may be used.

2. The zymotic chamber;

   The chamber is so constructed that permits as much oxidation as possible to take place in course of fermentation. The use of thermophilic fermenter is made freely in case it is necessary.

3. The products;

   The content of the chamber is taken out after the temperature falls down and becomes constant, on average, it requires about three weeks. The compost thus produced seems to be well fermented as that produced by an ordinary method of composting which requires much longer time. The chemical composition of the product varies as the initial materials which are put into the chamber vary. An average of some samples were produced from rice straw, barley straw (fresh

(8) Symposium on "Soil Deterioration", J. Amer. Soc. of Agronomy, **18**, 2, 1926.
(9) O. Schreiner, ibid, p. 121.

and some used once on roof), rice hasks, and weeds, gave the following composition.

	Percentage.
Total nitrogen,	2.00
" potassium,	1.44
" phosphates,	0.85

IV. Summary and Conclusions.

1. In the field of dynamic studies of organic matter especially of cellulose in agriculture, more extensive as well as intensive research investigation should be carried out.

2. The thermodynamical study in the general microbial processes specially on the soil microorganisms should be investigated in order to obtain better knowledge of soil fertility.

3. The thermophilic cellulose fermenter seems to act vigorously on the cellulose in course of composting as well as on highly refined cellulose in culture medium.

4. A specially constructed zymotic chamber seems to aid in producing the desirable compost out of various waste meterials in comparatively short time without the aid of cattle.

5. Such method of composting may be employed in a large scale as a process of disposing the waste materials in city as well as on the farm.

STUDIES ON THE RELATION OF BLOOD CONSTITUENTS AND FLACHERIE IN SILK-WORMS.

By Otomatsu Fujii.

(Received Feb. 22nd., 1926)

I. Quantitative Changes of the Blood Constituents During the Development of Silk-Worms.

1. The main purpose of this research is to investigate the causes of flacherie in the silk-worm, from the biochemical point of view. It is a well known fact that flacherie always appears at some definite periods, as for example one or two days after the fourth sleep and at the most active period

of feeding in the fifth age. So that, as a preliminary test, the author carried out a series of experiments to discover the changes occurring in some of the blood constituents during the development of the silk-worm.

In the blood constituents, total N, protein N, organic base N, amino acid N, P, Cl, Mg, K, and Ca were determined and also Ph was investigated. As the result of these experiments it became clear that the concentration of these constituents generally increases in the blood according to the increase of the amount of food digested, whilst the amino acid N alone decreases in the fifth age.

2. The author proposed a certain modification of Folin and Wus' method (J. Biol. Chem., **38**, 81, 1919) for the determination of nitrogen in the blood and discussed the sources of errors in the method.

II. On the Cause of Flacherie.

Flacherie, a disease of the silk worm, has been investigated by Pasteur, Vernon, Omori, Honda, Chigasaki etc. from the bacteriological point of view, and many species of bacteria have been isolated from the blood and the intestines of the diseased bodies, but further investigations have shown that these bacteria could not multiply in the blood or intestines of the healthy worms, and many trials have failed to produce this disease in the worms by feeding them with mulberry leaves on whose surface bacteria had been artificially smeared. On the other hand it has been reported by Cuboni, Voglino, and Sawamura that these bacteria are always found on the natural mulberry leaves. Hitherto the failure in hygienic cares, such as temperature, moisture and the method of rearing, were accounted as the cause of flacherie, but it was shown by Okushi (Report of Kumamoto Sericultural Experimental Station V, I, No. 3) that the variation of temperature and moisture in the natural state have no such effect on the worms.

The author, therefore, attempted to ascertain the cause of this disease from the biochemical viewpoint, and first of all studied the quantitative difference in some of the blood constituents between healthy and diseased bodies. According to the results of these experiments the amounts of protein N, amino acid N, Ca, Mg and Cl are greatly diminished in the blood of diseased bodies, as compared with those in the normal blood, and this abnormal condition agrees with that which arises in the healthy worms of the fifth age when they had been starved for forty eight hours. Such starved worms do not show any sympton of flacherie. But, when these starved worms were fed with mulberry leaves on whose surface some of the bacteria (or the blood of diseased worms) were smeared, about 90 % of them developed the full

symptoms of flacherie.

From the above results, it seems therefore that the infection with the bacteria alone can not be the cause of the disease, but that the disturbances in the digestive organs must go first and give chance to the rapid multiplication of bacteria, once infected, in the intestinal canal and the blood. The fact that the respective bacteria are always present on the mulberry leaves also confirms this conclusion.

THE SEPARATION OF DIBASIC AMINO ACIDS BY ELECTROLYSIS.

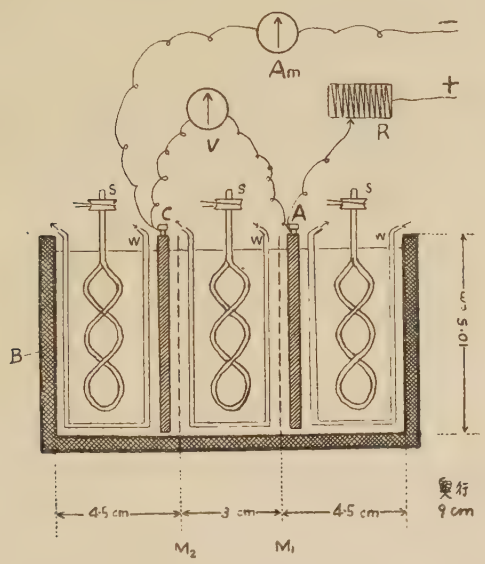
Tarō Noguchi.

(Received March 21st., 1926)

Experiments were carried out with the neutral barium salt solution of both pure glutaminic acid and aspartic acid, placing one of these solutions in the center of a three compartment cell and distilled water in the end compartments as shown in the figure, using carbon electrodes. The operation was made in various conditions, and the results obtained were as follows: Almost no destructive decomposition occurred when the current density at the anode surface did not exceed 0.25 amp. per 100 sq. cm. If the concentration of the amino acid wandered in the anodic compartment was kept under 0.5 % and the temperature in each compartment was maintained at 25°C by means of the water coolers, 90–95 % of the sample substance in the center compartment migrated through the membrance to the anode. In this case, a parchment paper membrane on the cathode side and a gelatin membrane on the anode side were found most suitable for keeping the solution in the center compartment neutral. Both the glutaminic and aspartic acids obtained in the anodic compartment were purified and analyzed.

By using the above method aspartic acid was separated from such a rejected liquid, which had been produced abundantly in our laboratory by hydrolyzing the protein of the soy-bean with sulphuric acid, removing the glutaminic acid present as calcium salts, and subjecting the resulting liquid to the butyl alcohol extraction. Thus 6.4 liters of the remaining liquid of the butyl alcohol extraction were electrolyzed, using a somewhat larger apparatus,

and 75 g. aspartic acid were obtained after 36 hours of electrolysis. The acid was then recrystallized and analyzed.



B. Bath (Cell)
A. Anode
C. Cathode
M_1 Gelatine membrane
M_2 Parchment paper
S. Stirrer
W. Water cooler
V. Voltmeter
Am. Ammeter
R. Resistance

STUDIES ON ACIDS FORMED BY RHIZOPUS SPECIES. PART II.

FORMATION OF ETHYL ALCOHOL FROM TARTARIC OR FUMARIC ACID.

By Teizo Takahashi, Kinichiro Sakaguchi and Toshinobu Asai.

(Received July 15th., 1925)

Further five species of Rhizopus of various origin were studied. One of them, Rh. japonicus Saito, formed mainly fumaric acid with trace of lactic acid, while Rh. shangheiensis Yamazaki gave both these acids in a reversed proportion. The other three species, Rh. nodosus Yam., Rh. Batatas Nakazawa, Rh. Tritici Saito formed both lactic and fumaric acids.

Lactic acid formed by these strains was *l*-lactic acid as we have mentioned in the previous report.

In the volatile products there were found beside ethyl alcohol both formic and acetic acids. The quantitative determination of acetic acid was made of this distillate after the destruction of formic acid by K–bichromate and sulphuric acid. The quantity of formic acid was calculated from the difference of the total acidity and that due to the acetic acid.

In a previous report the presence of malic acid was mentioned from the result of qualitative test. This acid has now been isolated and accurately identified by Dakin's[1] method. Cinchonin–*l*–malate thus obtained melted at 197–198°C (uncorr.), as given by Dakin.[2]

The results of the analyses are shown in the following table :–

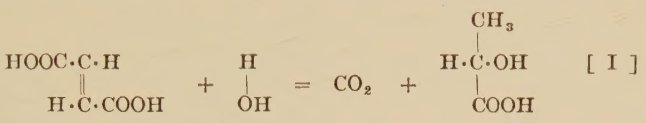
Species of Rhizopus.	R. japonicus	R. nodosus	R. Batatas	R. Tritici	R. shanghaiensis
Weight of fungus in 1 L. medium.	1.109 gr.	1.320	1.268	1.455	1.002
Sugar consumed.	38.4 gr.	47.5	46.8	37.8	54.0
Acidity of medium expressed in c.c. of $^1/_{10}$n NaOH to neutralize 10 c.c. of it.	14.4c.c.	22.2	24.6	18.6	21.6
Quantity of acid ppted as Pb-salt in the residue of ether extract expressed in c.c. of $^1/_{10}$n NaOH.	32.8c.c.	6.8	3.3	24.2	4.9
Quantity of volatile acid from 1 L. medium expressed in c.c. of $^1/_{10}$n NaOH. Formic acid.	1.0c.c.	7.8	2.0	13.4	2.3
Quantity of volatile acid from 1 L. medium expressed in c.c. of $^1/_{10}$n NaOH. Acetic acid.		12.2	8.0	11.6	1.7
Alcohol formed (wt. %)	0.67%	0.16	0.29	0.54	0.23
Acid in ether extract from 1 L. medium. Fumaric acid.	6.07 gr.	0.105	0.059	0.041	trace
Acid in ether extract from 1 L. medium. Lactic acid.	trace	17.1 gr.	18.0	13.9	16.5
Zn–lactate Water of crystallisation.		13.05%	13.14	12.91	13.21
Zn–lactate $[\alpha]_D^{20}$		+ 6.75°	+ 7.1	+ 6.81	+ 6.85
Remarks	Ba-salt, soluble in 80% alcohol, obtained from the ether extract was 0.03 g. Malic acid obtained as cinchonin-salt was 0.02 g.				Ba-salt insoluble in 80% alcohol obtained from the ether extract weighed 0.36g.

The formation of lactic acid and alcohol from fumarate was affirmed in in the culture of Rh. G. 36 in the medium consisting of: water 100 c.c., peptone 0.3 g. and K–fumarate 2.5 g. besides mineral matters. Lactic acid amounting to 0.063 g. was determined by Ripper's method.[3] The quantity of ethyl alcohol was too small to be determined quantitatively althongh it

(1) and (2). Dakin: Journal of Biol. chem. Vol LIX, No 1, p. 7. 1924.

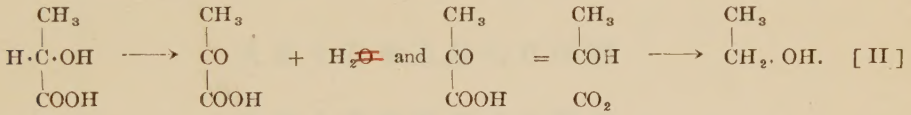
(3) Ripper: Bioch. Zeit. **42**, S. 91–104, 1912.

gave iodoform when treated by iodine and sodium hydroxide. The change of fumaric acid into lactic acid may be shewn in accordance with equation :-



[I]

As to the production of ethyl alcohol the most probable explanation is the intermediate formation of pyruvic acid from lactic acid, as already proved by Kayser[4] in the case of yeasts.



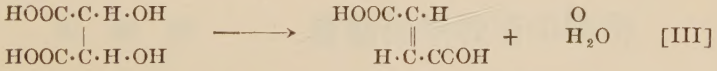
[II]

The change of tartarate into fumarate and alcohol was confirmed by the culture of Rh. Oryzea in the medium consisting of: water 1000 c.c., K_2HPO_4 0.15 g., KH_2PO_4 0.15 g., $MgSO_4$ 0.1 g., $CaCl_2$ 0.1 g., Fe_2Cl_6 and NaCl trace, Na or K-tartarate 20 g., peptone either 1 or 3 gr., or some times 2 g. and $CaCO_3$ 20 g.

Fumaric acid isolated from this culture melted at 279°C (uncor.) in a sealed tube and gave a characteristic colour reaction found by the authors.[5] Its silber salt dried at 110°C was analysed and gave the following data :-

Subst. taken.	AgCl.	Ag	as	$C_4H_2O_4Ag_2$
0.2485 g.	0.2242 g.	Found		65.57 %
		Calcul.		65.44 %

Its dimethyl ester melted at 100-101°C. The production of the fumaric acid from tartaric acid may be expressed by the following mode :-



[III]

The formation of ethyl alcohol by this fungus seems to take place most reasonably in accordance with the equations I and II. If we accept ~~ethes~~ the old theory that lactic acid is an intermediate product of alcoholic fermentation is proved in this case too, as in the case of yeast done by Kayser.[4]

(July 10, 1925).

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(4) Kayser : Comp. R. Tome 176. No. 22. p. 1663. 1923.

(5) Authors method will be published later.

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